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Lopinavir Plasma Concentrations and Virological Outcome with Lopinavir-Ritonavir Monotherapy in HIV-1-Infected Patients

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There is significant intra- and intersubject variability in lopinavir (LPV) plasma concentrations after standard dosing; thus, this prospective study was conducted to determine whether low plasma LPV concentrations could be associated with virological outcome throughout lopinavir-ritonavir maintenance monotherapy (mtLPVr) in the clinical practice setting. If this hypothesis would be confirmed, LPV drug monitoring could improve the efficacy of mtLPVr regimens. Patients with previous virological failure (VF) on protease inhibitor-based regimens were also included if the genotypic resistance tests showed no major resistance mutation associated with reduced susceptibility to lopinavir-ritonavir. VF was defined as 2 consecutive determinations of HIV RNA levels of >200 copies/ml. Efficacy was analyzed by per-protocol analysis. Plasma LPV trough concentrations were measured by high-performance liquid chromatography using a UV detector. A total of 127 patients were included (22% with previous failure on protease inhibitors). After 96 weeks, the efficacy rate was 82.3% (95% confidence interval [CI_{95}], 75.3 to 89.3%). Virological efficacy was independent of LPV plasma concentrations even when LPVr was given once daily. An adherence of <90% (HR, 4.4 [CI_{95}, 1.78 to 10.8; $P = 0.001$]) and the presence of blips in the preceding 12 months (HR, 3.06 [CI_{95}, 1.17 to 8.01; $P = 0.022$]) were the only variables independently associated with time to VF. These findings suggest that the LPV concentrations achieved with the standard doses of LPVr are sufficient to maintain virological control during monotherapy and that measurement of LPV concentrations is not useful for predicting virological outcome. Tight control of viral replication in the previous months and strict adherence throughout the mtLPVr regimen could improve the virological efficacy of this maintenance regimen.

There is significant controversy regarding ritonavir-boosted protease inhibitor (PI) monotherapy as a maintenance therapeutic strategy (1–5). Notwithstanding, a considerable proportion of patients maintained an undetectable viremia on lopinavir-ritonavir maintenance monotherapy (mtLPVr) and could benefit from a simpler regimen without nucleoside analogues or other antiretroviral drugs. Moreover, lack of adherence has been indicated as the main reason for virological failure (VF) in different studies (6–13).

Moreover, there is significant intra- and intersubject variability in LPV plasma concentrations after standard dosing, determined largely by variability in drug absorption, cytochrome P450 metabolism, plasma protein binding, and drug transporter activity, which may affect the disposition of the drug (14, 15). As LPV plasma concentrations and the genotype inhibitory quotient have been related to virological efficacy in experienced patients on LPVr-based regimens (16–19), in this study, we tested whether low plasma LPV trough concentrations contribute to VF throughout the mtLPVr treatment period. The confirmation of this hypothesis would demonstrate that LPV therapeutic drug monitoring could be useful in improving the efficacy of LPV when prescribed as a monotherapy.

MATERIALS AND METHODS

Study population and design. From April 2009 to April 2010, adult HIV-1-infected patients who started a regimen of mtLPVr at our outpatient clinic were consecutively included in this observational, prospective, open-label study. The LPVr dosing regimen (400 mg of LPV and 100 mg of ritonavir twice daily or 800 mg–200 mg once daily) was selected by the patients' physicians. All subjects had plasma HIV RNA levels of <50 copies/ml for at least 6 months. Patients with VF while on a PI-containing regimen were also included in the study when genotypic resistance tests showed no major or ≤3 minor resistance mutations associated with reduced susceptibility to LPVr according to 2008 International AIDS Society criteria (20). No inclusion restrictions were made regarding CD4 cell counts, hepatitis C virus (HCV) coinfection, abnormal laboratory parameters, or the presence of blips (transitory episodes of detectable plasma HIV RNA viral loads preceded and followed by a plasma viral load of <50 copies/ml without changes in antiretroviral treatment) during the previous 12 months. mtLPVr was not prescribed in cases of pregnancy, hepatitis B virus coinfection, or concomitant use of drugs with potential major interactions with LPVr pharmacokinetics, as recommended in the LPVr prescribing information (21). The study was designed and conducted according to the principles contained in the Declaration of Helsinki and approved by the Ethics Committee for Clinical Research of the Hospital Universitario Virgen del Rocio. All patients provided informed consent.

Endpoints, follow-up, and assessments. The main objective was to correlate plasma LPV levels with virological efficacy at 48 and 96 weeks, with VF defined as either (i) two consecutive viral load measurements of >200 copies/ml, (ii) a unique HIV RNA measurement of >200 copies/ml if followed by a loss of follow-up, or (iii) the reintroduction of nucleos(t)ide analogues for any reason. A cutoff level of 200 copies/ml was chosen because it is a more accurate measurement of VF than a lower cutoff value (22, 23). Because this was a pharmacological study, the main efficacy

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analysis was performed on the per-protocol population in which missing data or changes caused by toxicity were censored. Patients were classified according to their plasma HIV RNA levels throughout the follow-up period as follows: (i) a continuous viral load of <50 copies/ml (there were no differences in any variables between patients with continuous undetectable viral loads and those with HIV RNA levels of between 20 and 50 copies/ml); (ii) blips, as defined above; (iii) intermittent viremia, defined as episodes of detectable plasma HIV RNA viral loads of >50 copies/ml without meeting blip or VF criteria; and (iv) VF, as described above.

Secondary outcomes included virological efficacy according to an intention-to-treat analysis, where regimen failure was defined as the combination of VF and/or switching or stopping of treatment for any reason. Patient assessment was performed based on quarterly visits during 2 years, including the assessment of adverse events (AEs), analytical profiles, flow cytometric counts of CD4 cells/μl, and plasma HIV-1 RNA levels measured by PCR (COBAS AmpliPrep/COBAS TaqMan HIV-1 test, version 2.0). AEs and abnormal laboratory findings were evaluated according to a standardized toxicity grade scale (AIDS Clinical Trials Group). Genotypic resistance tests were performed on subjects with VF, as allowed by viral load levels. Patients who missed two consecutive scheduled visits were considered "loss of follow-up."

Blood sampling and determination of lopinavir concentrations. Blood samples for LPV determinations were drawn 12 or 24 h (±15 min) after the previous LPVr dose according to the dosing regimen (otherwise, blood samples were discarded) and processed within an hour after collection. Plasma was separated and stored at −80°C until assayed. LPV concentrations were measured by a high-performance liquid chromatography assay using a UV detector, according to a validated method (24), with accuracy and precision of 100% ± 15% and <10%, respectively. The assay was linear over the range of 0.08 to 10 μg/ml for plasma concentrations and validated for a concentration range of 0.123 to 10 μg/ml. The lower limit of quantification was 40 ng/ml. The rate of recovery of LPV from human plasma was 98.2% ± 2.66%. The mean intra- and interassay coefficients of variation (CVs) for plasma samples were 3.1 and 5%, respectively.

Statistical analysis. Categorical variables were compared by using the Student *t* test or the Mann-Whitney nonparametric test, according to their distribution. Intrasubject variability in drug concentrations was assessed by measuring the CV of all the available values from each patient throughout the follow-up period. Intersubject variability was calculated by using the CV for the geometric mean (GM) of the available values from each subject. Differences in LPV plasma concentrations between periods with viral loads of <50 and >50 copies/ml were analyzed by the Wilcoxon signed-rank test for patients who did not have continuous viral loads of <50 copies/ml. The relationships between VF and different variables were assessed by the chi-square test or Fisher's exact test for qualitative variables and Spearman's rank correlation coefficients for quantitative variables.

Time-to-event analyses were performed by using Kaplan-Meier survival curves and the log rank test. A Cox proportional-hazards model was used to assess the relationship of different variables with time to VF, using a stepwise selection procedure in which variables associated with *P* values of ≤0.05 remained in the model. Statistical calculations were performed with Statistical Product and Service Solutions software (v. 19.0; SPSS, Chicago, IL, USA).

RESULTS

Characteristics of the study population. A total of 127 patients were consecutively included in the study, whose main baseline characteristics are shown in Table 1. The LPVr dosing regimens consisted of 400 mg–100 mg twice daily (b.i.d.) for 113 patients (89%) and 800 mg–200 mg once daily (q.d.) for the remaining 14 patients (11%). Thirty-four patients (26.8%) had experienced a previous VF while on protease inhibitors (22 patients with nonboosted PI and 12 patients with ritonavir-boosted PI, including 5 patients who had VF on LPVr). Genotype information prior to mtLPVr treatment was available for 54 patients, for whom the following mutations in the protease gene were observed: L10I/V (*n* = 6), K20M/R (*n* = 3), D30N (*n* = 4), L33I/V (*n* = 2), M36I/L (*n* = 7), M46I/L (*n* = 4), F53L (*n* = 1), I62V (*n* = 1), G73S (*n* = 1), L63P (*n* = 30), A71V/I/T (*n* = 16), G73S (*n* = 1), V77I/S (*n* = 8), I85V (*n* = 1), N88D/S (*n* = 3), and L90M (*n* = 4). Thus, 21 (16.5%), 15 (11.8%), and 2 (1.5%) subjects showed 1, 2, and 3 minor resistance mutations associated with reduced susceptibility to LPVr, respectively.

TABLE 1 Baseline patient characteristics[a]

Parameter	Value (*n* = 127)
No. (%) of males	103 (81.1)
Mean age (yr) (range)	45 (29–75)
Mean wt (kg) (range)	68.5 (40.5–100)
No. (%) of patients with risk factor for HIV	
Previous i.v. drug use	66 (52.0)
Heterosexual/homosexual	54 (42.5)
Other	7 (5.5)
No. (%) of patients on methadone treatment	19 (15.0)
No. (%) of patients reporting active illegal drug use	10 (7.9)
Mean nadir CD4 cell count (cells/μl) (range)	129 (1–541)
No. (%) of patients of clinical category C	33 (26.0)
No. (%) of patients with chronic hepatitis C	71 (55.9)
No. (%) of patients with cirrhosis	16 (12.6)
Mean no. of previous ART regimens (range)	3 (1–9)
Mean time of previous ART (mo) (range)	91 (14–231)
No. (%) of patients with previous VF on PIs	34 (22.6)
No. (%) of patients with previous VF on PI-ritonavir	12 (10.2)
Mean time of HIV RNA level of <50 copies/ml (mo) (range)	38 (6–149)
No. (%) of patients with blips in the previous 12 mo	18 (14.2)
Mean CD4 cell count (cells/μl) (range)	581 (94–1,356)
No. (%) of patients with prior ART	
Lopinavir-ritonavir	57 (44.8)
Saquinavir-ritonavir	63 (49.6)
Atazanavir-ritonavir	3 (2.3)
Efavirenz	4 (3.1)
Analogues	
Tenofovir + emtricitabine	71 (55.9)
Abacavir + lamivudine	31 (24.4)
Other combinations	25 (19.6)
No. (%) of patients taking LPVr 400 mg–100 mg b.i.d./no. (%) of patients taking LPVr 800 mg–200 mg q.d.	113 (91.0)/14 (11.0)

[a] ART, antiretroviral treatment; VF, virological failure; PI, protease inhibitor; q.d., once daily; i.v., intravenous.

Efficacy and safety. At 48 and 96 weeks of treatment, the efficacy rates were 95% (95% confidence interval [CI_{95}], 91.1 to 98.9%) and 82.3% (CI_{95}, 75.3 to 89.3%), respectively, based on on-treatment analysis and 84.3% (CI_{95}, 78.3 to 90.1%) and 68.5% (CI_{95}, 60.7 to 76.3%), respectively, based on intention-to-treat analysis (Fig. 1). Thus, 40 patients (31.5%) experienced treatment failure at week 96, with VF being the cause of 20 of these failures (15.7%). Among those with VF, we also included 2 patients with nonconfirmed viremias of 325 and 423 copies/ml, followed by loss of follow-up, and 6 patients who added 2 nucleos(t)ide analogues to the LPVr treatment due to 2 positive viremias of <200 copies/ml.

Plasma HIV RNA amplification was achieved in 10 out of the

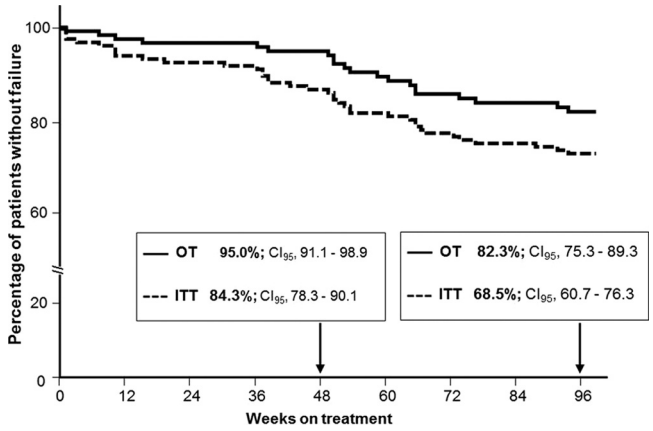

FIG 1 Proportion of patients without virological failure (continuous line) and without protocol-defined treatment failure (dashed line) through week 96. OT, on-treatment analysis; ITT, intention-to-treat analysis.

20 patients at VF. Six of them exhibited a wild-type virus, three exhibited new minor resistance mutations associated with reduced susceptibility to LPVr (A71T, L76V, L10I, K20R, and M46I), and only one patient exhibited a major mutation in the protease gene (V32I). Three months after VF, 18 of these 20 patients had undetectable HIV RNA levels due to either the addition of 2 analogues to their treatment ($n = 13$) or continued mtLPVr ($n = 5$). The remaining two patients were lost after VF.

Other reasons for treatment failures were AEs in 15 patients (diarrhea, 8 patients; grade 2 to 3 dyslipidemia, 5; rash, 1; increased glucose levels in patients suffering from diabetes mellitus, 1; death not related to study drugs, 2), loss of follow-up, or treatment dropout ($n = 2$). Only 5 subjects (8.9%) and 2 subjects (3.6%) without chronic hepatitis shown increased aminotransferase levels of grades 1 and 2, respectively, throughout the follow-up in any determination. Likewise, among those with chronic hepatitis or cirrhosis ($n = 70$), these figures were 26 (38%) for grade 1, 4 (5.6%) for grade 2, and 1 (1.4%) for grade 4, being transient and improving without treatment modification in every case. Figure 2 shows the lipid profiles of patients throughout the mtLPVr regimen. After 96 weeks of mtLPVr, the median changes in total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides were 19 mg/dl (interquartile range [IQR], −8 to 37 mg/dl), 2 mg/dl (IQR, −5 to 9 mg/dl), 8 mg/dl (IQR, −9 to 28 mg/dl), and 33 mg/dl (IQR, −18 to 96 mg/dl), respectively. The median increases in CD4 cell counts from baseline to weeks 48 and 96 were 43 cells/μl (IQR, −80 to 192 cells/μl) and 63 cells/μl (IQR, −68 to 182 cells/μl), respectively, which were inversely proportional to baseline CD4 counts.

Lopinavir plasma concentrations. A total of 728 LPV determinations were performed on samples from 123 patients throughout the follow-up period, with a median of 7 plasma samples per patient (IQR, 3 to 8; range, 1 to 9). The LPV minimum concentration of drug in serum (C_{min}) was higher for the 400-mg–100-mg b.i.d. dosing (5.91 μg/ml [IQR, 4.50 to 7.59 μg/ml; range, 0.11 to 14.64]) than for the 800-mg–200-mg q.d. regimen (1.99 μg/ml [IQR, 1.23 to 3.33 μg/ml; range, 0.79 to 5.54 μg/ml]) ($P = 0.002$). The intrasubject variability was lower for the b.i.d. dosing

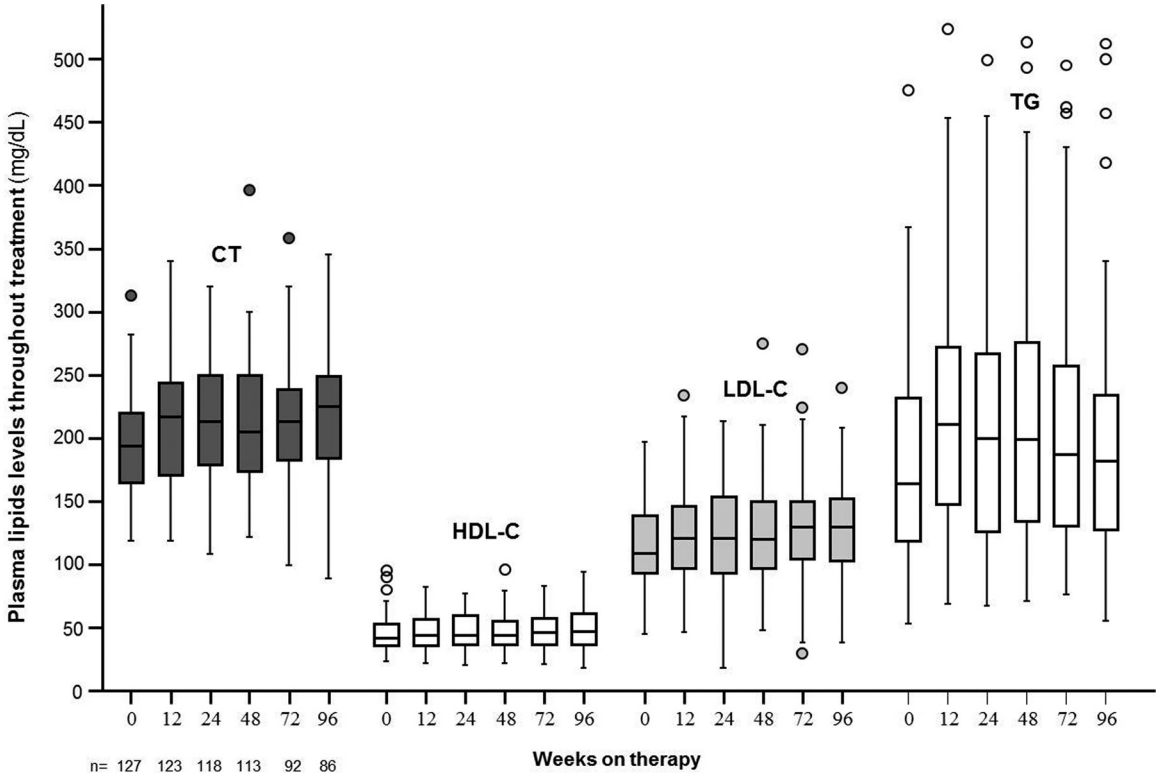

FIG 2 Change in lipid plasma levels (mg/dl) throughout the follow-up period. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

TABLE 2 Plasma LPV C_{min} as a function of virological outcome during follow-up[a]

Virological outcome	Mean LPV C_{min} (μg/ml) (IQR; range) during period of HIV RNA level of:	
	<50 copies/ml	>50 copies/ml
C < 50 ($n = 64$)	5.83 (4.48–7.65; 0.22–14.64)	
Blips ($n = 24$)	5.06 (3.56–7.78; 0.86–10.55)	4.56 (3.53–7.34; 0.40–14.12)
IV ($n = 16$)	5.48 (3.86–6.45; 0.34–7.72)	5.98 (3.70–8.23; 1.07–12.03)
VF ($n = 19$)	6.53 (4.29–7.69; 2.59–10.58)	4.95 (2.83–8.05; 0.60–12.76)

[a] Shown is a comparison of LPV concentrations during periods with HIV RNA levels of <50 versus >50 copies/ml or at virological failure. C < 50, continuous viral load of <50 copies/ml; IV, intermittent viremia; VF, virological failure.

regimen (34% [IQR, 19 to 45%; range, 4 to 132%]) than for the q.d. dosing regimen (53% [IQR, 37 to 82%; range, 34 to 109%]) ($P = 0.001$) for the 106 patients with more than two LPV determinations. The intersubject variabilities were 41.6% and 49.9% for the b.i.d. and q.d. regimens, respectively.

No relationships between plasma LPV concentrations and weight ($r^2 = 0.008$; $P = 0.51$) or body mass index ($r^2 = 0.0016$; $P = 0.486$) were observed, and there were no differences in LPV plasma concentrations with respect to gender or the presence of cirrhosis.

LPV plasma C_{min} values were similar between patients with continuous viral loads of <50 copies/ml and those who exhibited blips, intermittent viremia, or VF during the follow-up period. In addition, no significant differences were observed in LPV plasma concentrations between the periods of viral loads of <50 and >50 copies/ml in the last three groups (Table 2).

Variables associated with virological responses. The variables associated with VF in the bivariate analysis were as follows: adherence of <90% (30.8% versus 2.3%; $P < 0.001$), previous VF on IP/rtv (33.3% versus 8.7%; $P = 0.028$), the presence of blips in the previous 12 months (33.3% versus 7.3%; $P = 0.005$), and a viral load of <50 copies/ml for <24 months (20% versus 6.9%; $P = 0.033$). In contrast, neither VF with nonboosted PI, the presence or number of minor resistance mutations associated with reduced susceptibility to LPVr, dosing regimen (b.i.d. or q.d.), LPV plasma concentrations, CD4 cell counts (actual or nadir), nor the presence of chronic hepatitis was associated with VF.

In the Cox proportional-hazards model, only an adherence of <90% (HR, 4.4 [CI_{95}, 1.78 to 10.8; $P = 0.001$]) and the presence of blips in the previous 12 months (HR, 3.06 [CI_{95}, 1.17 to 8.01; $P = 0.022$]) were independently associated with time to VF. Moreover, there was no relationship between these two variables (Spearman rho correlation coefficient, 0.072; $P = 0.421$).

DISCUSSION

To our knowledge, this is the first prospective study that has assessed the efficacy of mtLPVr in the usual clinical practice setting, without the careful selection that a randomized clinical trial requires. Moreover, in contrast to previous studies, patients with a history of VF while on a PI-based regimen were enrolled, and there was no previous period in which tolerance to LPVr was determined before the start of monotherapy. Despite these facts, the virological efficacy was similar to that observed in clinical trials of mtLPVr (6, 7, 9–13). It is noteworthy to mention that neither a history of VF on an unboosted or ritonavir-boosted PI-containing regimen nor the presence of minor resistance mutations to LPVr nor the once-daily administration of LPVr had negative influences on the virological outcome. Moreover, the very low incidence of resistant mutations in the protease gene in VF and that viral suppression could easily be achieved by adding nucleos(t)ide analogues or even continuing the use of mtLPVr and improving adherence are issues to highlight. Likewise, it is worth to note the low incidence and grade of the AEs, which motivated a treatment switch as well as the good liver safety profile in a population of which more than 50% were affected by chronic hepatitis C or cirrhosis.

Surprisingly, against what might be expected, we have found no relationship between LPV concentrations and virological outcome. On one hand, this suggests that the LPV concentrations achieved with the standard doses of LPVr are enough to maintain virological control during monotherapy, even with LPV plasma C_{min} values lower than that recommended for triple therapy in subjects without resistance mutations associated with reduced susceptibility to LPVr (1 μg/ml) (2). On the other hand, our data suggest that measurement of LPV concentrations is not useful for predicting virological outcome. There are several plausible explanations for the lack of this relationship, including that LPV C_{min} was not the most correct pharmacodynamic parameter to evaluate its activity, as has been observed in highly antiretroviral agent-experienced subjects (16, 18, 25), or differences in susceptibility to LPV among the various viral isolates, although no subjects had major resistance mutations associated with reduced susceptibility to LPVr. However, we believe that the most plausible explanation might be the "white-coat compliance" phenomenon, in which adherence considerably improves even in subjects with poor adherence just prior to a study visit, especially if they know that pharmacokinetic sampling will be performed (26). This conclusion is supported by the finding that LPV concentrations were similar regardless of the virological outcome. Similar data were also observed in a subanalysis of the M03-613 study, in which the LPV C_{trough} and area under the concentration-time curve (AUC) were estimated by using Bayesian methods (27). In fact, it has been estimated that noncompliant subjects on an LPVr-based regimen need as few as 1 to 3 days of good adherence to achieve LPV concentrations in the range observed for fully compliant subjects (28).

The only independent variables associated with VF in our study were an adherence of <90% and the presence of blips during the 12 months prior to the start of mtLPVr. Whereas poor adherence has been pointed out as the main risk factor for loss of virological control in patients receiving mtLPVr in previous clinical trials (7, 29), the presence of blips before the start of mtLPVr has not been assessed as a risk factor for VF. This result suggests that strict control of viral replication is required before considering an mtLPVr regimen. In contrast, neither a history of VF on an unboosted or ritonavir-boosted PI-containing regimen, the presence of minor resistance mutations, the CD4 count nadir, the once-daily administration of LPVr, nor the length of virological suppression before the start of monotherapy was associated with VF.

The main limitation of our study is the way in which we evaluated adherence. The use of hospital pharmacy records might not accurately reflect how medication was taken, and this my be the main reason for the lack of a relationship between LPV plasma levels and virological outcomes.

In summary, the results of an mtLPVr maintenance regimen

are as good in clinical practice as in clinical trials, even in patients with previous VF on protease inhibitor-based regimens but without major resistance mutations associated with reduced susceptibility to LPVr. The LPV concentrations achieved with the standard doses of LPVr are enough to keep virological control during monotherapy. Our data suggest that therapeutic drug monitoring of lopinavir concentrations is not useful for improving the efficacy of this regimen. A tight control of viral replication in the previous 12 months and a strict adherence throughout the mtLPVr regimen are two key factors that could improve the virological efficacy of this maintenance regimen.

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